

### Carbon-13/Carbon-12 Ratio Is Relatively Uniform Among Honeys

**Abstract.** *The variability of the carbon-13/carbon-12 ratio in honeys was evaluated preliminary to use of the ratio to detect the addition of high-fructose corn syrup to honey. Eighty-four honey samples representing 34 states and including 37 floral types from 17 plant families were analyzed. The mean value of the per mil increment in carbon-13 ( $\delta^{13}\text{C}$ ) for all samples is  $-25.2$  per mil, and the coefficient of variation is 3.7 percent. This is the smallest variation yet encountered for a honey constituent or physical property. The range and magnitude of the values suggest that the floral sources are  $\text{C}_3$  plants.*

We have undertaken to develop methods to detect the undeclared addition to honey of high-fructose corn syrup (HFCS), a new, highly refined syrup now produced in large quantity (1). Its similarity to honey in major components and in minor oligosaccharides and the great variability of the composition of honey make most approaches unfruitful.

When inorganic carbon is converted to living matter during photosynthesis, an isotope effect produces differences in the  $^{13}\text{C}/^{12}\text{C}$  ratios among the various reservoirs of the carbon cycle (2). The organic compounds of cells invariably have a slightly lower  $^{13}\text{C}/^{12}\text{C}$  ratio than the carbon dioxide and carbonate of the environment. Isotope variations among these reservoirs result from differences in chemical and physical properties of molecules containing different isotopic species (3).

In surveys of many plant families,

Bender (4) and Smith and Epstein (5) demonstrated large differences in  $\delta^{13}\text{C}$  values (6) among plants. Bender, in analyzing the family Gramineae, found that plants that initially fix carbon dioxide via the  $\text{C}_4$  dicarboxylic acid pathway have  $\delta^{13}\text{C}$  values in the range  $-10$  to  $-20$  per mil and those which follow only the  $\text{C}_3$  cycle have  $\delta^{13}\text{C}$  values of  $-22$  to  $-33$  per mil. Thus  $\text{C}_3$  plants fractionate atmospheric carbon dioxide to a greater extent than do  $\text{C}_4$  plants. Families that show crassulacean acid metabolism also have many examples of high  $\delta^{13}\text{C}$  values. Smith and Epstein looked at many plant families and found several to be high in  $^{13}\text{C}$  content ( $\delta^{13}\text{C}$  range,  $-5.6$  to  $-18.6$  per mil), while those lower in  $^{13}\text{C}$  content ( $\delta^{13}\text{C}$  range,  $-23.2$  to  $-34.3$  per mil) comprise the bulk of the plant kingdom, lacking the  $\text{C}_4$  pathway of carbon dioxide fixation.

Smith and Epstein (5) suggested that

**Table 1.** Carbon-13/carbon-12 ratios of selected U.S. honeys. Carbon isotope ratios were determined by Geochron Laboratories Division of Krueger Enterprises Inc. (Cambridge, Mass.) on an AEI MS-20 double-collecting, 180°-sector mass spectrometer with a dual-capillary inlet. Usual corrections were applied to the measured differences, including any zero enrichment in the capillary inlet system, valve mixing between sample and standard valves, tailing of major onto minor peak signals, and contribution of  $^{17}\text{O}$  to the mass 45 signal. Samples were combusted in purified  $\text{O}_2$  at about 850°C and the gases were recirculated over CuO at 850°C for 10 minutes; water and  $\text{CO}_2$  were frozen, excess  $\text{O}_2$  was removed, and  $\text{CO}_2$  was transferred to a sample flask for analysis. Results are expressed in  $\delta^{13}\text{C}$  units (6). The overall reproducibility for the system was stated to be 0.3 per mil in the ratio; however, five duplicate samples of honey agreed within 0.1 per mil. Interlaboratory crude oil reference samples also agreed within 0.1 per mil overall.

Family	Floral type	Number of samples	$\delta^{13}\text{C}$ (per mil)
Anacardiaceae	<i>Schinus molle</i> (pepper tree)	1	-25.0
Aquifoliaceae	<i>Ilex glabra</i> (gallberry)	1	-25.6
Compositae	<i>Centaurea solstitialis</i> (star thistle)	1	-26.3
Cornaceae	<i>Nyssa ogeche</i> (tupelo)	1	-26.0
Cyrtillaceae	<i>Cyrilla parvifolia</i> (titi)	1	-24.2
Euphorbiaceae	<i>Sapium sebiferum</i> (tallow tree)	1	-26.4
Labiatae	<i>Salvia</i> spp. (sage)	1	-24.2
Leguminosae	<i>Glycine soja</i> (soybean)	2	-26.8
Leguminosae	<i>Medicago sativa</i> (alfalfa)	10	-25.2
Leguminosae	<i>Melilotus</i> spp. (sweet clover)	2	-26.4
Leguminosae	<i>Trifolium</i> spp. (clover)	11	-25.6
Magnoliaceae	<i>Liriodendron tulipifera</i> (tulip tree)	1	-25.3
Malvaceae	<i>Gossypium hirsutum</i> (cotton)	1	-24.7
Onagraceae	<i>Epilobium angustifolium</i> (fireweed)	1	-25.4
Palmae	<i>Sabal</i> spp. (palmetto)	1	-24.7
Polygonaceae	<i>Fagopyrum esculentum</i> (buckwheat)	1	-25.2
Rosaceae	<i>Rubus</i> spp. (blackberry)	1	-26.1
Rutaceae	<i>Citrus</i> spp. (orange, grapefruit)	3	-23.4
Tamaricaceae	<i>Tamarix gallica</i> (tamarisk)	1	-25.1
Tiliaceae	<i>Tilia americana</i> (basswood)	1	-25.6
	Unclassified natural season blends	15	-25.2
	Honeydew honey	4	-24.5

one could easily distinguish whether a particular brand of sucrose was obtained from sugarcane ( $\text{C}_4$  plant) or from sugar beet ( $\text{C}_3$  plant), a distinction not possible using classical chemical methods. Nissenbaum *et al.* (7) used this approach to detect the addition of sucrose to Israeli citrus juice. They found the  $\delta^{13}\text{C}$  values of citrus juice solids (-24.3 to -25.0 per mil) to be very similar to that of beet sugar (-24.3 per mil) but very different from that of cane sugar (-12.2 per mil). Unfortunately, beet sugar is used almost exclusively in Israel so no practical application of this method was realized. Recently, however, Hillaire-Marcel *et al.* (8) utilized this method to detect the adulteration of maple syrup with cane sugar. The  $\delta^{13}\text{C}$  for cane sugar was  $-11.47 \pm 0.5$  per mil, for Vermont maple syrup  $-24.05 \pm 0.45$  per mil, and for Quebec maple sugar  $-23.54 \pm 0.40$  per mil. They reported the ability to detect adulteration at the 10 percent level.

Listings of  $\delta^{13}\text{C}$  values (4, 5) show rather wide ranges for some nectar-producing plants: two Compositae (5) have values of -24.2 and -34.3 per mil, and among five Euphorbiaceae, a range from -15.3 to -29.4 per mil is recorded. To evaluate this approach for the detection of corn products in honey, information is needed on the variability of  $\delta^{13}\text{C}$  among honeys of different origin.

We have collected directly from U.S. beekeepers nearly 500 certified samples of honey from the 1974 and 1975 crops. For 84 representative samples selected from these, we have obtained  $\delta^{13}\text{C}$  values. Samples from 34 states included 37 named floral types from 17 plant families. The criteria for sample selection were such that (i) the geographic distribution was proportional to that of honey production, (ii) for several floral types and blends produced in greatest amount, samples were selected from as wide an area as possible, (iii) at least one example of each commercially significant type was included, (iv) natural mixtures representing production for the entire season were selected from many areas, and (v) honeydew honeys, identified by positive optical rotation, were included. Table 1 summarizes the results for at least one sample from each family. The identification of floral source by the beekeeper is shown. Values for each sample with data on floral source and area of production will appear elsewhere (9). For the objective of this work, the important aspect was that the samples represent honey types commonly available in quantity. In view of the remarkable uniformity of the data, the need for unequivocal floral source identification appears secondary. For a study of the relatively small differences among honey

types and areas of production, examination of hand-collected nectar would be the obvious choice.

The mean  $\delta^{13}\text{C}$  for all samples was -25.2 per mil; the coefficient of variation was 3.7 percent. This is the smallest variation yet encountered for any honey constituent or physical property. An analysis of 490 samples for major components (10) showed the smallest coefficient of variation (fructose) to be 5.4 percent; most minor constituents varied between 25 and 75 percent, and for trace components a 10- to 100-fold variation was sometimes seen (11).

Even though honeybees may range for miles around the colony, with access to many nectar plants, nectar from the major sources appears to greatly dilute any contributions from high- $^{13}\text{C}$  plants. It is perhaps noteworthy that the three samples with the highest  $^{13}\text{C}$  contents (*Prosopis glandulosa*, Hawaii, -22.5; *Acacia greggii*, Arizona, -22.8; and season blend, Arizona, -22.7 per mil) originated in xeric environments, which have been suggested to favor adaptation to a high  $^{13}\text{C}/^{12}\text{C}$  ratio (5). The almost exclusively carbohydrate nature of honey must also contribute greatly to the uniformity of the values. The ranges of  $\delta^{13}\text{C}$  values for the constituents of single plants (12) are wider than the range found here for all the samples examined. Details of the application of these and other data to the detection of HFCS addition to honey will appear elsewhere (9).

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#### References and Notes

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6. The  $\delta^{13}\text{C}$  value is defined by

$$\delta^{13}\text{C} (\text{per mil}) = \left[ \frac{(^{13}\text{C}/^{12}\text{C})_{\text{sample}}}{(^{13}\text{C}/^{12}\text{C})_{\text{standard}}} - 1 \right] \times 10^3$$

The reference standard is Pee Dee belemnite (PDB) carbonate.

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13. We thank J. G. Phillips for statistical counsel.